Proceedings of the American Academy of Arts and Sciences

VOL. 77, No. 7, P. 235–253—JUNE, 1949

A "FREE MANOMETER" TECHNIQUE FOR USE WITH THE WARBURG APPARATUS

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Received March 29, 1949

INTRODUCTION

The Warburg apparatus, as commonly employed, is based upon a gas reaction occurring within a closed system of known and constant volume. Pressure changes are read directly on the open side of the manometer, the temperature being held constant. Under these conditions, assuming that the gas laws apply, the pressure change is a *linear function* of the volume (or moles) of gas evolved or taken up. This function is known as the "vessel constant" and depends upon gas volume, fluid volume, temperature, solubility of the reacting gas, atmospheric pressure, and manometer fluid density.[1] The linearity of the relationship *appears* to require that the gas volume be held constant, and this can only be achieved by repeatedly levelling the fluid on the vessel side of the manometer to a predetermined mark. That the levelling procedure is inconvenient, and productive of certain errors and limitations, will be evident to those who have employed the apparatus extensively.

In the modification to be described, the manometer fluid is not levelled during a reaction; i. e., both pressure and volume are allowed to vary. The fluid on the *vessel side* of the manometer is set initially to the manometer midpoint (150 mm.) and subsequent readings are then made on this same arm of the manometer. The manometer fluid adjustment is never touched again after the initial setting. A reciprocal and equal change occurs in the fluid levels in the two arms as a reaction proceeds, but the fluid in the open arm is ignored. Thermobarometer corrections are made as usual (for certain limitations see p. 243) and the readings multiplied by vessel constants in the customary fashion, to obtain micromoles (or cu. mm.) gas at NTP.

In justification of this procedure we shall show that in the Warburg apparatus a constant volume is *not* the necessary condition for a linear function relating pressure change to evolved (or absorbed) gas; that, on the contrary, when both pressure and volume are allowed to vary, a linear function is still obtained, within the limits of accuracy

* A substantial part of this work was done during tenure of a Lalor Fellowship, and expenses for equipment were defrayed by a generous grant from the Abbott Laboratories, North Chicago, Illinois.

of the method (1%); and that, consequently, the levelling operation can be omitted.

This "free manometer" technique offers the following points of difference from the constant volume method:

(1) Since nothing need be adjusted during a reaction, automatic recording—as by a time-controlled camera—becomes feasible.

(2) The error introduced by the levelling procedure itself is eliminated. We refer to the small differences in pressure readings upon repeated levelling of the meniscus, the result of unavoidable inaccuracies in the performance of this operation.

(3) The error introduced by levelling immediately before a reading is eliminated. We refer here to the transient pressure change resulting from a sudden change in the gas space as the fluid is levelled, and before the new gas-fluid equilibrium is established. Although the errors cited here and in the paragraph above are small, their reality can be readily confirmed.

(4) Readings can be made more rapidly and more frequently, since the time consumed by the levelling operation is eliminated. This is especially welcome when one wishes to follow the first few minutes of a reaction, or when a reaction is proceeding so rapidly that considerable change occurs while the usual seven manometers are being read. If one reads to no better accuracy than 1 mm., it is perfectly satisfactory to read the manometers in motion.

(5) The capacity of the standard manometer is substantially increased while its sensitivity is correspondingly reduced. Thus on the one hand it becomes possible to measure a total gas change three to four times as great as by the standard method, without resetting the manometer; on the other hand small quantities of gas cannot be detected as well or measured as accurately. Whether capacity is preferable to sensitivity will depend upon the circumstances, and there are certain applications for which the former proves more desirable than the latter. Two such cases where we have found the method decidedly useful are studies of the time course of enzyme-substrate reactions and measurement of substrate oxidation by bacteria in the presence of a high endogenous respiration. We have calculated the vessel constants for both methods and find it convenient to use the free manometer technique as a matter of course, reserving the constant volume method for those occasions when a high sensitivity is desired.

(6) As against the advantages cited must be weighed the drawback of a somewhat more complex equation for the calculation of vessel constants. Besides calibrating the vessel volume one must also determine the linear volume of the manometer (cu. mm. per mm.).

This, however, need not involve an additional operation if one follows the calibration method suggested by Burris,[2] (p. 50), filling the manometer first to a point above, and then to a point below the 150 mark. If one determines the constants empirically, these problems are, of course, irrelevant. As will be seen shortly, the constants are not quite so readily transformed for changed conditions of temperature or fluid volume as in the constant volume method.

General Theory of the Method

A. *The relation of pressure change to volume change.*

Although pressure and volume both vary, a fixed relationship is imposed by the fact that manometer fluid is neither added nor removed during a reaction. Thus (see figure 1), $\Delta p = 2\Delta h$ and $\Delta v = M\Delta h$, where M, the manometer factor, is cu. mm. per mm. manometer.

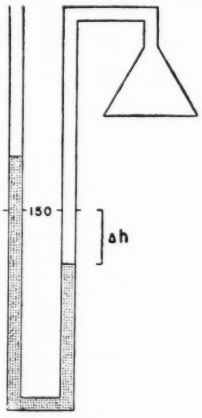

FIGURE 1.

B. *The complete equation.*

We shall develop the vessel constant equation for the case of a reacting gas, x, and an inert gas, z, both present. The full equation is not linear, but we shall show that by making certain legitimate simplifications it can be transformed to linear form.

Let P_0 = initial total pressure (atmospheric), expressed in mm. manometer fluid = 10,000 mm. when manometer fluid density is 1.033.

P_w = vapor pressure of water at temperature T (absolute).

P_x, P_z = initial partial pressures of the gases.

P_x', P_z' = final partial pressures of the gases.

α_x, α_z = solubility coefficients (moles per cu. mm. at P_0, T).

V = initial gas space (cu. mm.).

V_f = fluid volume (cu. mm.).

M = manometer factor (cu. mm. per mm.).

R = gas constant (8.21×10^8 cu. mm. atm. [mm. manom. fluid] $\times$ deg.$^{-1}$ $\times$ mole $^{-1}$).

Δv = change in gas volume.

Δp = change in total pressure.

Δh = change in manometer level on one side of manometer.

For the inert gas z

$$\text{Initially, moles } z \text{ in gas phase} = \frac{P_z V}{RT}$$

$$\text{moles } z \text{ dissolved} = \frac{V_f \alpha_z P_z}{P_0}$$

$$\text{Total moles } z = P_z \left[\frac{V}{RT} + \frac{V_f \alpha_z}{P_0} \right]$$

$$\text{Finally, moles } z \text{ in gas phase} = \frac{P_z' (V + \Delta v)}{RT}$$

$$\text{moles } z \text{ dissolved} = \frac{V_f \alpha_z P_z'}{P_0}$$

$$\text{Total moles } z = P_z' \left[\frac{V + \Delta v}{RT} + \frac{V_f \alpha_z}{P_0} \right]$$

Since z is inert, total moles z remains constant. Thus, equating, and substituting $\Delta v = M \Delta h$:

$$P_z' = P_z \left[\frac{VP_0 + V_f \alpha_z RT}{VP_0 + V_f \alpha_z RT + P_0 M \Delta h} \right] \tag{1}$$

For the reacting gas x, the same expressions will apply except that now total final moles will be equal to the sum of total initial moles plus males evolved (or absorbed). Thus:

$$P_x' \left[\frac{V + \Delta v}{RT} + \frac{V_f \alpha_x}{P_0} \right] = P_x \left[\frac{V}{RT} + \frac{V_f \alpha_x}{P_0} \right] + x$$

or

$$P_x' = P_x \left[\frac{VP_0 + V_f \alpha_z RT + \dfrac{P_0}{P_x} \cdot RTx}{VP_0 + V_f \alpha_z RT + P_0 M \Delta h} \right] \tag{2}$$

Now to simplify the following operations, let

$$\begin{aligned} VP_0 &= A \\ V_f RT &= B \\ P_0 RT &= C \\ P_0 M &= F \end{aligned} \qquad \begin{aligned} A + B\alpha_x &= X \\ A + B\alpha_z &= Z \end{aligned}$$

Equations (1) and (2) then become:

$$P_z' = P_z \left[\frac{Z}{Z + F \Delta h} \right] \tag{1a}$$

$$P_x' = P_x\left[\frac{X + \dfrac{Cx}{P_x}}{X + F\Delta h}\right] \tag{2a}$$

Initially: $P_0 = P_x + P_z + P_w$

Finally: $P_0 + \Delta P = P_x' + P_z' + P_w$

$$\Delta P = (P_x' - P_x) + (P_z' - P_z) = 2\Delta h$$

Then from (1a) and (2a):

$$\frac{Cx}{\Delta h} = P_xF + P_zF \cdot \frac{X}{Z} \cdot \frac{\left(1 + \dfrac{F}{X}\Delta h\right)}{\left(1 + \dfrac{F}{Z}\Delta h\right)} + 2(X + F\Delta h)$$

or

$$\frac{x}{\Delta h} = \frac{X}{C}\left[\frac{P_xF}{X} + \frac{P_zF}{Z}\left(\frac{1 + \dfrac{F}{X}\Delta h}{1 + \dfrac{F}{Z}\Delta h}\right) + 2\right] + \frac{2F\Delta h}{C} \tag{3}$$

If, in equation (3), the terms in Δh *on the right* could be neglected, we would be left with a linear equation of the usual form, $x/\Delta h = k$, the entire term on the right becoming the vessel constant. To perform this simplification one must show that the term in Δh within the brackets is essentially equal to unity, and that dropping the final term $2F\ \Delta h/C$ does not introduce an error as great as 1% in the vessel constant. At this point we shall state as a fact that this can be done; the reader is referred to Appendix A for details.

Making these simplifications and substituting the original symbols, we obtain for the vessel constant:

$$k = \underbrace{\left[\frac{\mathbf{V}P_0 + V_f\alpha_xRT}{P_0RT}\right]}_{\textit{Term a}}\underbrace{\left[\frac{P_xP_0\mathbf{M}}{\mathbf{V}P_0 + V_f\alpha_xRT} + \frac{P_zP_0\mathbf{M}}{\mathbf{V}P_0 + V_f\alpha_zRT} + 2\right]}_{\textit{Term b}\qquad\qquad\textit{Term c}} \tag{4}$$

It should be observed that the only terms which vary from vessel to vessel are those in boldface, namely **V** and **M**.

C. *Simplified forms of the equation.*

If equation (4) be rewritten in such a way as to separate the major

terms containing $V_f\alpha$ from those containing V, we obtain a useful form which lends itself to further simplification:

$$k = \frac{2V}{RT} + \frac{V}{RT}\left[\frac{P_xP_0M}{VP_0 + V_f\alpha_xRT} + \frac{P_zP_0M}{VP_0 + V_f\alpha_zRT}\right] + \frac{2V_f\alpha_z}{P_0} + \frac{V_f\alpha_z}{P_0}\left[\frac{P_xP_0M}{VP_0 + V_f\alpha_xRT} + \frac{P_zP_0M}{VP_0 + V_f\alpha_zRT}\right] \quad (4a)$$

In the case of a single gas (e. g. oxygen uptake in an atmosphere of pure oxygen) it will be clear that the terms in z drop out entirely. Furthermore, for any gases of very low solubility (nitrogen, oxygen) the terms $V_f\alpha RT$ become entirely negligible compared with VP_0 and can therefore be neglected. Equation (4a) then reduces to the extremely simple form:

$$k = \frac{2V + M(P_0 - P_w)}{RT} + V_f\alpha_x\left[\frac{2V + M(P_0 - P_w)}{VP_0}\right] \quad (5)$$

The first term in equation (5) is the intrinsic vessel constant (k_0) in the absence of any fluid. Once calculated, it serves as a basis for subsequent transformations to conform to changed conditions. For example, since temperature appears nowhere but in this term, recalculation of constants for changed temperature requires only that k_0 at temperature T_0 be multiplied by the ratio T'/T_0, a procedure which is independent of the gases involved. In the measurement of oxygen uptake (or evolution) the vessel constant is simply k_0 regardless of fluid volume, the solubility being so low that *the entire second term of equation* (5) *becomes negligible.**

Recalculation of constants for different fluid volumes is more involved since every change in V_f is accompanied by an opposite change in V; except for cases (mentioned above) where the second term can be dropped, the entire equation will have to be recalculated.

The principles outlined here are essentially those developed for the constant volume method by Umbreit,[2] (p. 53). Here one must always be certain that the assumptions leading from equation (4a) to equation (5) are valid for the particular experimental conditions obtaining. Of the gases most often measured, *oxygen, as a rule, will yield valid constants by equation* (5); *carbon dioxide usually will require* (because of its high solubility) *that equation* (4) *be used in full.*

* This and similar statements apply, of course, only to usual magnitudes of the various terms in the equations. They are not to be taken in any more general sense but should be re-examined for unusual gas volumes, temperatures, manometer bores, etc.

For the case of two reacting gases (e. g. oxygen absorbed, carbon dioxide evolved), one can proceed through the same steps as previously, to obtain equation (3) in terms of x and y,

$$\frac{x}{\Delta h}+\frac{y}{\Delta h}\cdot\frac{X}{Y}\left(\frac{1+\frac{F}{X}\Delta h}{1+\frac{\dot{F}}{Y}\Delta h}\right)=\frac{X}{C}\left[\frac{P_xF}{X}+\frac{P_yF}{Y}\left(\frac{1+\frac{F}{X}\Delta h}{1+\frac{F}{Y}\Delta h}\right)+\frac{P_zF}{Z}\left(\frac{1+\frac{F}{X}\Delta h}{1+\frac{F}{Z}\Delta h}\right)+2\right]+\frac{2F\Delta h}{C}$$

This is simplified as before, to give:

$$x+\frac{X}{Y}\cdot y=\frac{X}{C}\left[\frac{P_xF}{X}+\frac{P_yF}{Y}+\frac{P_zF}{Z}+2\right]\cdot\Delta h$$

For the special case of R. Q. experiments, let us assume x to be carbon dioxide and y to be oxygen, and the respiration to take place in air. With x absorbed by alkali, y is determined in the usual way:

$$y=k_y\cdot\Delta h$$

Now when *both* gases are reacting, and since the initial $P_x=0$, it can be shown that

$$x=\frac{X}{Y}\left[k_y\cdot\Delta h-y\right]$$

or, substituting symbols, and simplifying the denominator:

$$x=\frac{VP_0+V_f\alpha_xRT}{VP_0}\left[k_y\cdot\Delta h-y\right] \qquad (6)$$

It is clear from equation (6) that the constants for carbon dioxide are not required, x being obtained indirectly from k_y and the known evolution of y, separately determined.

D. *The thermobarometric correction.*

In the constant volume method the effects of temperature variation and changes in atmospheric pressure are reflected in an equal change in the level of all manometers, regardless of gas space, fluid volume, temperature, or the particular gases present. In the free manometer

method this is not strictly true, although it will usually be possible to consider it so without significant error.

It can be shown (see Appendix B) that for a temperature change ΔT,

$$\Delta h = \frac{1}{T\left(\frac{2V + P_0M}{VP_0}\right)} \cdot \Delta T$$

If we make the reasonable assumption that the temperature variation will never exceed 0.2°, the *total* Δh due to ΔT will be about 2.5 mm., and the *differences* between several vessel-manometer combinations will be entire negligible.

Unfortunately, the changes of level due to small variations in atmospheric pressure cannot always be disposed of so simply. It can be shown (see Appendix B) that for a change in atmospheric pressure ΔP_0,

$$\Delta h = \frac{V}{2V + P_0M} \cdot \Delta P_0$$

Here differences between vessels become significant (> 0.5 mm.) when $\Delta P_0 > 10$ (or about 0.8 mm. mercury). It should be noted, however, that as compared with the constant volume method, the actual Δh due to ΔP_0 is exceedingly small.

In experiments of several hours duration we have found it unusual for the thermobarometer to vary more than 5 mm. (a variation which corresponds to about 15 mm. in the constant volume method). Should this figure not be exceeded, the thermobarometric correction may be treated in the customary way without detectable error. For greater variations a correction term may be introduced:

$$\Delta h_x = \left[\frac{\frac{2V_1 + P_0M_1}{V_1}}{\frac{2V_x + P_0M_x}{V_x}}\right] \cdot \Delta h_1 \qquad (7)$$

where Δh_1 is the change in level of the thermobarometer, and Δh_x is the appropriate correction to be applied to any particular vessel. It should be noted that the terms which appear in this correction factor must all have been calculated previously in determining the constant for each vessel. Use of this thermobarometric correction factor, which is independent of temperature, fluid volume, or the nature of the gases present, permits accurate readings despite large changes in atmospheric pressure.

It will be observed from the above equations that the magnitude of manometer excursion due to thermobarometric changes, and consequently of differences in manometer responses, can be minimized by employing a small vessel with a manometer of large bore. The smaller vessel also increases the general sensitivity of the method, thus curiously *increasing* the manometer excursion in response to gas changes while at the same time *decreasing* its excursion in response to thermobarometric changes. A large manometer bore, on the other hand, markedly *decreases* the general sensitivity of the method, while very large bores (of the order of 2 mm.) introduce errors into the method. The practical expedient, therefore, is to use, if possible, *small vessels with small-bore manometers;* to choose such combinations of vessels and manometers as will tend to equalize the $M : V$ ratios; and to select as thermobarometer that combination having an intermediate $M : V$ ratio.

Unlike customary procedure, the thermobarometer here should contain approximately the same amount of fluid as the other vessels, and should be calibrated for V and M.

Application of the Method

A. *Practical procedures.*

We have shown that from a theoretical standpoint, within an error of 1%, the free manometer method will give readings which are proportional to the amount of gas liberated or absorbed. The vessel constant can be calculated (as illustrated in the next section) from the same data required in the constant volume method, once the *linear volume* of the manometer has been determined to an accuracy of about 1%. Or the vessel constant can be found empirically by measuring the release of a known amount of gas.

No special apparatus is required. Small vessels (about 10 cc.) are preferable to large. Manometers of small bore (about 1 mm.) are also preferred. If the inside diameter is greater than 1.5 mm. significant errors may be introduced. The bore of a set of manometers ought not to vary widely. Precautions should be taken to prevent fluid being inadvertently added or removed during the progress of a reaction.

Small thermobarometric changes (5 mm. or less) may be treated in the customary way. Whether large changes will be reflected equally in all vessels depends entirely upon the $M : V$ ratios for the various vessel-manometer combinations. Inspection of equation (7) will reveal the significance of the differences for any particular set of vessels and manometers. Thermobarometric changes exceeding 5 mm. in a period of several hours are rarely encountered in practice.

For *oxygen uptake*, with carbon dioxide absorbed, the simplest form of the vessel constant equation applies (equation (5) without the second term).

For *R. Q. determinations*, the carbon dioxide constant is not needed. Using the oxygen constant alone, the evolved CO_2 is determined from the oxygen uptake with CO_2 absorbed, and the *apparent* oxygen uptake with both gases present. (See equation (6).)

For CO_2 *evolution*, because of the high solubility, it is necessary to use the full equation (4). The simplified form, equation (5), gives constants that are too large by about 2%.

Ordinarily readings are started by closing the stopcocks and simply setting the fluid *in the vessel arm* of each manometer to 150. The manometers are then not touched again, and calculations are much simplified by the fact that all subsequent readings are substracted from 150. If, despite the increased capacity of the free manometer technique, it becomes necessary to reset the manometers, this can be done very simply. The stopcocks are opened momentarily to allow fluid to equalize in both manometer arms. It is immaterial whether this level is exactly 150 or (because drainage is incomplete) somewhat lower. The reading at the moment of resetting is called 150 and, provided drainage is complete by the time of the next reading, no error is introduced.

If a very large gas evolution or uptake is to be measured, the capacity can be doubled by setting to 150 with the stopcocks open, applying positive pressure to the appropriate arm of the manometers, and closing the stopcocks while fluid is thus displaced almost entirely into one manometer arm. The zero time reading will then be close to 300, or to 0, as the case may be, and the entire length of the manometer will be available for subsequent fluid excursion.

B. *Typical determination of a vessel constant.*

Vessel no. 2. Manometer no. 2.

Weight of mercury filling vessel to neck mark of manometer at 26°: 234.5 Gms. Calculated volume to neck mark: 17.33 cc. In this step the weighing need be done to no better than 0.1 Gm. Now filling the manometer with mercury from the neck mark to a point above and then below the 150 mark, we find:

Neck mark to 80.5 (23°) : 10.27 Gms. mercury.

Neck mark to 173.0 (23°) : 8.35 Gms. mercury.

Then 92.5 mm. mercury at 23° weighs 1.92 Gms. and $M = 1.54$ cu. mm. per mm.

Neck mark to 150 (by interpolation) : 0.65 cc.

Note that the weighings in this section must be to 0.01 Gm.

As a check on the above value of M, and in order to ascertain the constancy of the manometer bore, we ran a bit of mercury 50 to 200 mm. long into the manometer. By measuring its length at different portions of the manometer we could verify the uniformity of the bore; the length of the column never changed by more than 1 mm. and no systematic narrowing or widening of bore was ever noted. Thus:

189 mm. mercury at 23° weighed 3.92 Gms.
109 mm. mercury at 23° weighed 2.28 Gms.
From which $M = 1.54$.

It should be clear from the above data that an error in excess of 1–2% in M could not reasonably occur. It is demonstrated in Appendix C that for a manometer of this bore, an error of up to 4% would be quite tolerable.

The vessel constant for CO_2, for a fluid volume of 2.2 cc. and a temperature of 27° C. is calculated as follows, using equation (4)

Total volume to 150 mark: 17,980
V_f: 2,200
V: 15,780
M: 1.54
T: 300
P_0: 1.004×10^4 mm. manometer fluid (of density 1.030).
Gas mixture: 95% N_2 − 5% CO_2
P_w: 26.74 mm. mercury at 27° = 353 mm. manometer fluid.
P_{CO_2}: 484.
P_{N_2}: 9198.
R: 8.21×10^8
α_{CO_2}: 3.20×10^{-8} moles per cu. mm.
α_{N_2}: 6.22×10^{-10}

In the following, the lettered terms refer to equation (4), which is used in full.

VP_0	1.586×10^8
$V_f RT\alpha_{CO_2}$	0.174×10^8
$VP_0 + V_f RT\alpha_{CO_2}$	1.760×10^8
$P_0 RT$	0.248×10^{16}
Term (a)	7.09×10^{-8}
$P_{CO_2} P_0 M$	7.45×10^6
Term (b)	0.04
$P_{N_2} P_0 M$	1.416×10^8
$V_f RT\alpha_{N_2}$	0.003×10^8
$VP_0 + V_f RT\alpha_{N_2}$	1.589×10^8
Term (c)	0.89

(b) + (c) + 2 = 2.93
2.93 × (a) × 10^6 = 0.208 μM per mm. = k.

C. *Experimental confirmation of the method.*

The validity of the free manometer method was confirmed by direct quantitative recovery of weighed bicarbonate, and by simultaneous determinations of enzymatically-controlled acid production in bicarbonate medium (plasma cholinesterase plus acetylcholine), and of oxygen uptake by a bacterial suspension. Vessel volumes varied from 16.22 to 20.29 cc. and manometer bores from 1.37 to 1.99 mm. diameter (M from 1.48 to 3.12). Fluid volumes of 2.2 and 5.5 cc. were employed.

In all such replicate determinations the free manometer and constant volume methods were in satisfactory agreement. The actual data obtained in one such confirmatory experiment are presented in Table 1, which readily allows comparison of the capacity and sensitivity of the two methods.

Appendix A. *The simplification of equation* (3).

It will be recalled that the elimination from equation (3) of certain terms in Δh was a prime necessity in transforming a complex function to a linear equation which would yield an expression for the vessel constant. The justification for this transformation will now be presented. In the following, a change which introduces a net error of less than 1% into the vessel constant will be accepted. Where Δh appears it will be assigned its maximum possible value, 150, to bring out the greatest errors that may arise through the simplifications.

The manipulations performed on equation (3) involved the dropping of the terms in Δh from two places. First we shall consider the elimination of the term

$$\left[\frac{1+\frac{F}{X}\Delta h}{1+\frac{F}{Z}\Delta h}\right]$$

from within the brackets. Substituting the proper symbols makes it apparent that the resulting fraction

$$\left[\frac{1+\frac{P_0M}{VP_0+V_f\alpha_xRT}\Delta h}{1+\frac{P_0M}{VP_0+V_f\alpha_zRT}\Delta h}\right]$$

must practically equal unity unless α_x and α_z differ considerably from each other. For oxygen and nitrogen, for example, both solubilities

TABLE 1

Enzymatic hydrolysis of 17.7 μM acetylcholine bromide by cholinesterase, *simultaneously* determined by both methods. The first two columns under each method give actual readings in mm. The third columns give computed μM CO_2.

	Free Manometer			Constant Volume		
Vessel:	9	10	μM CO_2	12A	7	μM CO_2
Constant:	0.274	TB		TB	0.0640	
	Set at	Set at				
18′	150	150	0	148.5	169	0
20′	140.5	150.5	2.74	148.	210.	2.68
22′	132.	151.	5.20	147.5	246.	4.99
24′	124.	151.	7.40	148.	281.5	7.23
26′	114.	148.	9.32	158.	120.*	9.20†
28′	107.5	147.5	10.96	159.	148.	10.93
30′	102.5	148.	12.47	158.5	171.5	12.46
32′	98.	148.5	13.83	158.	191.	13.74
34′	94.5	148.5	14.80	157.	207.5	14.86
36′	92.	149.	15.62	156.	220.5	15.76
38′	90.	149.5	16.30	155.	229.	16.36
40′	89.	150.	16.70	154.	234.	16.75
Total Δh	61.0 mm.			226.5 mm.		
Extreme TB variation	3.5 mm.			11.5 mm.		
1 μM equals	3.7 mm.			15.6 mm.		
1 mm. equals	0.274 μM			0.0640 μM		

* Re-set manometer here. † Extrapolated value.

are so small and so similar that by no reasonable combination of other terms could the entire fraction be made as great as 1.01 or as small as 0.99. Thus the error could not exceed 1% even if this were the only term in the equation. As a matter of fact, the term under consideration is added to two others in equation (3) so that we are allowed an error in excess of 1% for the term itself. When the two gases are carbon dioxide and nitrogen, whose solubilities differ so greatly, one can no longer categorically dispose of the term as negligible. However

if we confine ourselves to reasonable magnitudes for the various terms it can still be shown that the one under consideration cannot exceed the limits indicated. For example, if $V > 5000$, $V_f = 2200$ (or 5500 when V is as large as 15,000), T about 300, the term is still between 0.99 and 1.00, provided M is less than 2. At larger total gas volumes M may be as large as 3 but when the actual manometer volume becomes a significant fraction of the total volume, the linearity of the equation no longer holds.

The same arguments apply to the elimination of the similar term in X and Y from the equation for two reacting gases and one inert gas.

The validity of dropping the final term $\frac{2F\,\Delta h}{C}$ depends primarily upon M. Substituting for the symbols, $\frac{2P_0M\,\Delta h}{P_0RT} = \frac{300M}{RT}$ for maximum Δh. R being constant, and T being subject to small differences (relative to its own magnitude), the numerical value becomes, approximately, $M \times 0.001 \times 10^{-6}$. Since the smallest value of the vessel constant may be of the order of 0.200×10^{-6} it follows that the error due to dropping this term may vary from 0.5% when $M = 1$, to 1.5% when $M = 3$. Both errors refer to extreme readings of the manometer; for readings below 150 the errors will, of course, be correspondingly smaller.

In summary, the two terms can be dropped without introducing a significant error. At full gas evolution (or absorption) the error just exceeds 1% if the manometer factor is as large as 3. It will now be understood why a large bore manometer is inadvisable. The equations will be most valid (i. e., reactions will run truly linearly) for small M, and a value between 1 and 1.5 should prove practical. This corresponds to a bore *diameter* between 1.1 and 1.4 mm., a range into which, fortunately, most manometers of recent make fall.

APPENDIX B. *Derivation of the thermobarometric correction.*

For change in T:

Initially $pv = NRT$

After ΔT, $\qquad (p + \Delta p)\,(v + \Delta v) = NR\,(T + \Delta T)$

Expanding, and dropping $\Delta p\,\Delta v$, $\Delta v = \dfrac{NR\Delta T - V\Delta p}{p}$

Substituting $\Delta v = M\Delta h$, $\Delta p = 2\,\Delta h$, and $p = P_0$

$$\Delta h = \frac{NR\Delta T}{P_0M + 2V}$$

But

$$NR = \frac{P_0 V}{T}$$

Thus

$$\Delta h = \frac{P_0 V}{T\,(2V + P_0 M)} \cdot \Delta T$$

For change in P_0:

Initially, inside vessel $pv = NRT$

After ΔP_0, $(p + \Delta p)\,(V + \Delta V) = NRT$

Expanding, subtracting, and dropping $\Delta p\,\Delta v$:

$$\Delta p = \frac{P_0}{V} \cdot \Delta V$$

Now the change in atmospheric pressure will equal the sum of the pressure change within the vessel and the pressure change shown on the manometer; or, $\Delta P_0 = \Delta p + 2\Delta h$. Substituting as above:

$$\Delta h = \frac{V}{2V + P_0 M} \cdot \Delta P_0$$

Net thermobarometer change:

$$(\Delta h)_{TOTAL} = \frac{P_0 V}{T\,(2V + P_0 M)} \cdot \Delta T + \frac{V}{2V + P_0 M} \cdot \Delta P_0$$

$$= \frac{V}{2V + P_0 M}\left[\frac{P_0}{T} \cdot \Delta T + \Delta P_0\right]$$

and since the term in the brackets applies equally to *all* manometers and vessels,

$$\Delta h_x = \left[\frac{\dfrac{2V_1 + P_0 M_1}{V_1}}{\dfrac{2V_x + P_0 M_x}{V_x}}\right] \cdot \Delta h_1$$

APPENDIX C. *The determination of M.*

To what accuracy must M be determined, and is the required accuracy obtainable by the usual calibration procedures? It is simplest to examine the question first from the standpoint of equation (5), although the same considerations apply to the full equation (4).

M only appears in the term $2V + M(P_0 - P_w)$ where any error will be reflected as an error of the same degree in the whole vessel constant. The problem is therefore to determine M so accurately that the error, ΔM, will be less than 1% of the entire expression in which M appears:

$$\Delta M < 0.01 [2V + M(P_0 - P_w)]$$

For $V = 1 \times 10^4$, $\Delta M < 0.03$ when $M = 1$; $\Delta M < 0.04$ when $M = 2$; and $\Delta M < 0.05$ when $M = 3$. The assumed value of V is a reasonable one; if V is larger the permissible error in M may be greater, and conversely. The required accuracy of determining M is given by $\Delta M/M$, which is 3%, 2%, and 1.7% respectively, when M equals 1, 2 and 3, at the assumed value of V.

We were concerned with the practical question whether a small-bore manometer could be calibrated with sufficient accuracy. A manometer of approximate bore 0.7 mm. was filled with columns of mercury 25 to 150 mm. long, the mercury weighed, and the operation repeated four times. The results were as follows (22°):

133.0 mm.	0.712 Gms.	0.00536 Gms. per mm.
25.0	0.134	0.00536
150.5	0.805	0.00535
151.0	0.811	0.00537
$M = 0.396$		Maximum error = 0.75%

In the case $M = 0.4$, the permissible error is 7% so that the accuracy obtainable is nearly ten-fold that required. The calibration of a manometer of larger bore having been demonstrated earlier, it is evident that the actual determination of M presents no practical difficulty.

Summary

1. A "free manometer" technique for use with the standard Warburg apparatus has been described, wherein the manometer fluid is not levelled. Although, as a consequence, both pressure and volume vary, the change in fluid level remains (within acceptable limits of error) a linear function of the quantity of gas evolved or absorbed.

2. The method opens the way to automatic recording of the progress of reactions. It also substantially increases the amount of evolved or absorbed gas that can be measured by a standard manometer.

3. The method has been derived in general theoretical terms, its practical application discussed, and its validity confirmed experimentally.

I am indebted to Dr. Paul Olum for helpful advice in connection with the mathematical treatment, and to my wife for suggestions and experiments bearing on the practical use of the method.

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